

Title: *CKM Glu83Gly is associated blunted creatine kinase variation, but not with myalgia*

Running title: *CKM Glu83Gly, creatine kinase and myalgia*

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## Abstract

**Background:** To test the association of a recently reported variant in the creatine kinase muscle gene, CKM Glu83Gly (rs11559024) with constitutive creatine phosphokinase (CK) levels, CK variation and inducibility. Given the diagnostic importance of CK in determining muscle damage we tested the association of the variant with myalgia.

**Methods and Results:** Meta-analysis between longitudinal cohort Genetics of Diabetes Audit and Research, Tayside Scotland (GoDARTS), minor allele frequency, MAF=0.02 and randomized clinical trial (JUPITER, MAF=0.018) was used to replicate the association with baseline CK measures. GoDARTS was used to study the relationship with CK-variability. Myalgia was studied in JUPITER trial participants. *Baseline and standard deviations of CK were on average 18% ( $P$ -value= $6 \times 10^{-63}$ ) and 24% ( $P$ -value= $\times 10^{-5}$ ) lower for carriers of the variant respectively. The variant was not associated with myalgia (OR=0.84;95%CI: 0.52,1.38).*

**Conclusions:** *This study highlights that a genetic factor known to be associated with constitutive CK levels is also associated with CK variability and inducibility. This is discussed in the context of evidence to suggest the variant has an impact on inducibility of CK by trauma through a previously reported case of a homozygous carrier. However, the lack of association between the variant and myalgia suggests that it cannot reliably be used as a biomarker for muscle symptoms.*

## Keywords

Biomarkers, Genetic Association Studies, Cardiovascular Disease, creatine kinase, muscle, adverse drug event, statin

## INTRODUCTION

Creatine phosphokinase also known as creatine kinases (CK) is an enzyme, 381 amino acids in length.<sup>1</sup> It catalyzes the reversible reaction that utilizes creatine to produce phosphocreatine and adenosine 5'-diphosphate (ADP) by the dephosphorylation of adenosine 5'-triphosphate (ATP). This is an exergonic reaction, and is important in the maintenance of energy homeostasis in all muscle tissues. CK is a dimer composed of two sub-units, CK-M and CK-B that combine to form three isoforms. The CK-MM isoform is found predominantly in skeletal muscle, CK-MB in the brain and cardiac muscle, and the CK-BB in smooth muscle. CK-MM makes up the majority of measured serum CK.<sup>2-4</sup> *CKM* encodes the CK-M subunit.

The enzyme creatine phosphokinase (CK) is a routine biochemical test performed in the clinical setting with widespread applicability. CK-M is used as a marker for tissue damage, muscle breakdown, muscular dystrophy<sup>5,6</sup>, infection<sup>7</sup>, acute kidney failure<sup>8</sup>, myocardial infarctions<sup>9-12</sup>, rheumatoid arthritis<sup>13,14</sup> and some diseases of the liver<sup>15-17</sup>. Notably, it is used as a marker of muscle damage or myopathy in adverse reactions to statins<sup>8,18,19</sup>. Additionally, CK-M has documented associations with hypothyroidism<sup>20</sup> and blood pressure<sup>21,22</sup>.

In 2014 Dubé *et al.* performed an original study in a Canadian population of statin users. They report a SNP rs11559024 (Glu83Gly) in *CKM* as being associated with serum CK levels. They found heterozygous carriers of the rare allele (Glu83Gly) had a mean CK level of 68.13 (SD 35.57) U/L compared to 119.32 (SD 84.74) for homozygous carriers of the common allele (Glu83Glu). They also report that statin dose and duration of use did not impact this association.<sup>23</sup> The MAF in the Canadian study was 0.010<sup>23</sup>. Kristjansson *et al.* replicated these findings in a GWAS performed on 63159 Icelanders with CK measurements. They report the main effect of the *CKM* Glu83Gly variant as being associated with serum CK ( $\beta = -0.446$ ,  $p$  value =  $1.8 \times 10^{-115}$ ). They also report that the variant was not associated with statin-related side-effects in a sub-cohort of approximately 8900 statin users. The MAF in the Icelandic cohort was 0.0215.<sup>4</sup>

Given the robust association and biologically apparent role of the gene in production of CK, we wanted to gain insight into the relationship between the variant and CK response. Since CK elevations in response to appropriate stressors is the clinically significant feature of the biomarker, we sought to understand the impact of the variant on inducibility of CK. The analytic cohort used was the GoDARTS study. GoDARTS is a rich source of data, combining complete electronic medical records including prescription information, all laboratory results from clinical visits from a cohort of 18190 individuals in Tayside, Scotland. Genetic information is available for a proportion of these individuals. Due to its longitudinal nature, the GoDARTS database has a median of 9 CK tests per individual. Therefore, it is the ideal template with which to examine intra-individual variability of CK by genotype. Further, since raised levels are used as markers of muscle-based symptoms, we sought to understand the relationship between the variant and the development of myalgia. If the variant confers no protective effect, this might provide a novel mechanistic rationale for a sub population of individuals presenting with statin intolerance or myalgia without raised CK levels.<sup>18</sup>

The findings of this analysis could impact the viability of CK as a reliable biomarker. We provide evidence to suggest that CK test results should be analyzed in the context genotypic data, especially for statin intolerance.

## MATERIALS AND METHODS

### Genotyping

Tayside Medical Ethics Committee approved the GoDARTS study and informed consent was obtained for all participants. The dataset contains complete electronic medical records, prescription information and laboratory results from 18,306 Scottish Caucasian individuals. In GoDARTS, genotype data for the *CKM* Glu83Gly variant was available for 6271 individuals, of whom 4578 were genotyped using the Human Exome - 12 VI\_A\_chip and 1693 using TAQMAN. The MAF of *CKM* Glu83Gly in the GODARTS cohort was 0.02. The JUPITER trial protocol was approved by the local institutional review board at each participating center. Genotyping for 8749 JUPITER trial participants was performed on the Omni1-Quad platform (Illumina, San Diego). The *CKM* Glu83Gly variant, rs11559024 was directly typed and MAF was 0.018.<sup>24</sup> The variant was in Hardy-Weinberg equilibrium in both cohorts.

### Creatine phosphokinase

Creatine kinase measures were gathered from the GoDARTS population where available. Test results from wards such as Accidents & Emergency (A&E), Cardiac Care, stroke, surgical wards and high dependency units were excluded for the analysis of baseline or un-induced CK levels with the variants. Individuals who had a history of thyroid disease, and those who had suffered a myocardial infarction (MI), kidney disorder, or had been involved in a serious accident in the 6 months preceding the test date were excluded from the analysis of baseline or un-induced CK with the variant.

For the analysis of CK variability, all measures were considered irrespective of referring centre and intra-individual standard deviations were computed for individuals with 3 or more CK tests. The upper limit of the normal range for women over 19 years of age is 120 IU/L and 180 IU/L for men of the same age as specified by the meta data documents provided by the Health Informatics Centre (Farr Institute). All clinical and biochemical testing in the GoDARTS cohort is performed at centralized laboratories in

Ninewells Hospital, Dundee, United Kingdom. The CK test results used for replication in the JUPITER trial were tested at baseline, when the population was treatment-naïve. The end products measured by the CK enzyme assay are provided in Electronic Supplementary Materials (Figure S1).

### Myalgia in JUPITER

The analysis in JUPITER focused on 4381 study participants randomized to receive statin treatment and 4368 who received the placebo.<sup>24,25</sup> Since the trial focused on the role of low-grade underlying inflammation (evidenced by high C-reactive protein levels), patients with inflammatory conditions such as severe rheumatoid arthritis, lupus, or inflammatory bowel disease were excluded, as were patients using immunosuppressant agents.<sup>25</sup> Myalgia was ascertained by physicians blinded to trial allocation arm. Only treatment-emergent adverse events were reported.<sup>26</sup> Myalgia was observed in 837 trial participants and was reported by Hsia et al. to be independent of their assigned therapy.<sup>26</sup>

### Statistical analysis

All statistical analyses for GoDARTS were performed in SAS 9.3 (SAS Institute, Cary NC). Plots were generated in R studio.<sup>27</sup> Statistical analyses in the JUPITER trial were performed using R.<sup>28</sup> Fixed effects meta-analyses were performed using the metafor package in the R studio environment.<sup>27–29</sup> The Cochran Q test was used to determine heterogeneity. Logarithmic transformations were applied to all CK levels to normalize their distribution. The first recorded CK measurement taken in an ambulatory setting for each individual was used to study the association between the variant and CK levels using linear regression. Intra-individual standard deviations were calculated for those with 3 or more CK measures. The association between intra-individual standard deviation and genotypes was tested using linear regression. The beta, standard error and  $R^2$  are reported. Binary logistic regression models and a survival analyses were used to test associations of the variant with myalgia in the JUPITER trial using R.<sup>28</sup>

## RESULTS AND DISCUSSION

### 1. Baseline characteristics of the GoDARTS study population

The average age was 66 years at the time of recruitment into the study (range 27 and 93); CK measures were available retrospectively and prospectively from recruitment date. Females comprised 40% of the study population. Certain factors that might be associated with CK levels such as age, sex, BMI, type 2 diabetes status, and creatinine levels were tested for association with the *CKM* Glu83Gly genotype, and none were found to be significant (see Table 1). Effect estimates of the genotype in analyses were therefore unaffected by the inclusion of these variables. Baseline features of the JUPITER population are described elsewhere.<sup>24–26</sup>

### 2. Association with baseline CK: GoDARTS and JUPITER

Creatine kinase levels were statistically significantly associated with the *CKM* Glu83Gly variant ( $n = 4598$ ,  $p$  value =  $2 \times 10^{-16}$ ) in the GoDARTS population. Carriers of the *CKM* variant (Glu83Gly:T/C) had mean CK of 86 ( $\pm 68$ ) compared 126 ( $\pm 82$ ) for homozygotes of the common allele as seen in Table 2. A boxplot of the difference in CK levels by genotype is presented in Electronic Supplementary Figure S2.

The association with the *CKM* Glu83Gly variant was replicated in the JUPITER trial ( $n = 8745$ ,  $p$  value  $< 2 \times 10^{-45}$ ). A meta-analysis with the GoDARTS cohort showed a highly robust association ( $\beta = -0.18$ ,  $p$  value =  $1 \times 10^{-63}$ ), and the test of heterogeneity was non-significant ( $p$  value = 1). A forest plot of the association is presented in Figure 1.

From the meta-analyses of the GoDARTS and JUPITER populations we conclude that carriers have 18% lower (log 10 transformed) CK on average, which translates to a 1.5 IU/L lower baseline CK level.

### 3. Association with CK variability

In order to assess if CK levels respond to stress differently by Glu83Gly genotype in *CKM*, we undertook an analysis of the intra-individual variation (represented by standard deviations) for individuals with 3 or more CK measures in the GoDARTS population ( $n = 3\,246$ ). The standard deviation of an individual's CK test results were

stratified by genotype as seen in Figure 2. We observe that Glu83Gly variant exerts a strong effect on the variability of CK measures in an individual (beta = -0.24%, p value  $2 \times 10^{-5}$ ). Carriers of the variant have a reduced range of variability in their measures. There is on average 24% reduction in the (log 10 transformed) standard deviation for carriers, indicating their CK levels do not always respond in a manner similar to non-carriers.

#### 4. Association with myalgia in JUPITER

Given the use of CK as a marker of muscle damage, especially for statin users, we examined if this variant, that is associated with blunted CK response was associated with the development of myalgia in a clinical trial. We observed no association between the variant and the development of myalgia in the JUPITER population (OR 0.84; 95% CI: 0.52, 1.38) p value = 0.5 (Kaplan-Meier plot presented in Figure 3). This analysis was conducted using the genotype as a dominant trait, since there were only 3 homozygous carriers of the variant. The analysis had 90% power to detect an effect of the size of the point estimate.<sup>30</sup> There was no association in analyses stratified by treatment allocation arms of the trial: rosuvastatin arm (OR = 0.64; 95% CI: 0.31, 1.29) or placebo arm (OR = 1.12; 95% CI: 0.57, 2.17).

## DISCUSSION

We report the replication of the association between the *CKM* Glu83Gly variant and constitutive CK levels using data obtained in a prospective cohort study (GoDARTS) and in the treatment-naïve population of a clinical trial (JUPITER). Further, we show that the variant is also associated with variability of CK levels. The use of standard deviations to calculate the variability gives us a conservative estimate of the effect, as contrasting the highest and lowest CK for each individual could also be used to assess the impact of the variant. The conclusions about the impact of the Glu83Gly variant in *CKM* on CK inducibility is further strengthened by the single individual in the GoDARTS cohort who was a homozygous carrier of the variant (Gly83Gly:C/C), presented as a case report by Wallace *et al.*<sup>31</sup> Details of the medical history are presented in Electronic Supplementary



Figure S3. During hospitalization for necrotizing fasciitis (event 1), a condition during which there is aggressive infection of the tissue and where CK levels could rise to  $> 600\text{IU/L}$ <sup>32</sup>, the patient's CK levels did not exceed  $10\text{IU/L}$ . In response to the subsequent development of gangrene (event 2) the patient underwent a debridement procedure, post-operatively CK levels were at a maximum of  $28\text{IU/L}$ . Later, the patient underwent a hemicolectomy for bowel cancer (event 3). The patient's pre- and post-operative CK levels remained relatively unchanged and in fact, seemed lower ( $34\text{IU/L}$  and  $25\text{IU/L}$  respectively). Notably the patient was a statin user who had undergone over 4 switches in statin therapy between 3 types of statins, namely pravastatin, atorvastatin and simvastatin. This switching is attributed to complaints of intolerance, however her CK measurements had been deemed normal by clinicians looking for evidence of statin-induced myopathy.<sup>31</sup>

In the case of the homozygous carrier of the variant we see the lack of CK response in conditions that would normally cause extremely high CK levels, such as severe tissue infection and surgical trauma.<sup>31</sup> Based on the CK assay that measures enzyme activity, we conclude that carriers of this *CKM* Glu83Gly variant are less likely to produce large quantities of measureable, and therefore, functioning CK in response to tissue damage.

Since the variant inhibits measureable CK levels from rising, potentially obfuscating the correct diagnosis, it might be essential to factor in the genotype of the individual before determining the validity or normalcy of the result. This is especially crucial since the genotype shows no association with myalgia, an outcome most commonly associated with elevated CK levels.

The diminished quantity of functional CK in the serum (see Electronic Supplementary FigureS1), would have to be compensated for by other mechanisms in the body to maintain energy homeostasis. One potential hypothesis revolves around the switch between aerobic and anaerobic metabolism. An oxygen debt created when there is insufficient phosphocreatine to make enough ATP needed during periods of high exertion. The process of energy generation then shifts from muscles to the liver (where

ATP is generated anaerobically via glycolysis and the Cori cycle, which delays the oxygen consumption process). Therefore, those with low CK activity (*CKM* carriers) might make this switch sooner, to maintain homeostasis.

The minor allele frequency for the *CKM* Glu83Gly variant in the GoDARTS population was 0.02, in JUPITER it was 0.018, similar to the European population in the 1000 Genome project (0.022). The Canadian (0.01) and the Icelandic (0.0215) cohorts also had a similar MAFs. This indicates that the populations under study were not suffering from participant selection bias. The frequency of the *CKM* variant in the Kenyan Masai population is 0.223.<sup>33</sup> This significant difference in the allele frequencies in the Masai is striking and warrants further investigation in other populations and for association with features of muscle or athletic performance. Undertaking whole-exome sequencing of homozygous carriers would enable us to determine if there are other rare variants in *CKM* that might be causative.

Statins are the most popular class of lipid-lowering medications. Intolerance to statins manifests as muscle symptoms, including myalgia and is observed in 10% of statin users. Intolerance results in poor compliance to medication, which in turn increases the risk of adverse cardiovascular events such as myocardial infarctions and strokes.<sup>34–36</sup> Definitions of statin intolerance in population-based studies hinge on the elevation of CK and, in some instances on the resolution of muscle-associated CK elevations upon the discontinuation of statin therapy.<sup>19,37,38</sup> The most established threshold for a “clinically relevant” CK elevation is 4 or more times the upper limit of normal (4 X ULN) and 10 X ULN.<sup>37</sup> However, as this study suggests, these outcomes are less likely to occur amongst carriers of the *CKM* variant. Indeed we note the under-representation of variant carriers in 231 clinically adjudicated cases of statin-induced myopathy of Caucasian ancestry in the PREDICTION-ADR study<sup>37</sup>, who met the CK elevation criteria and had been exome sequenced. However, due to the size of the study and the MAF of the variant, we are underpowered to determine if the difference is significant. We posit that the employment of traditional classifications would lead to the artificial enrichment of non-carriers of the *CKM* variant being classified as having statin-

induced myalgia, or myopathy. Indeed, this artefact is noted in the occurrence of CK above the ULN in GoDARTS (Electronic Supplementary Table S1).

As highlighted by Stroes *et al.*, in the European Atherosclerosis Society consensus panel statement on the impact of statin-associated muscle symptoms (SAMS) on statin therapy, often cases of SAMS are not accompanied by marked CK elevations.<sup>18</sup> Phillips *et al.* have reported cases of statin-associated myopathy, that were resolved on discontinuation and return upon re-challenge, that showed no elevations in CK.<sup>39</sup> The Journal for American Family Physician also highlights the potential for this to occur, concluding that pathologic evidence of myopathy might be present among patients with adverse muscle symptoms with normal CK levels.<sup>40</sup> This is congruent with the lack of association between this CK-associated variant and non-specific myalgia in this study.

It is of great clinical and scientific interest to understand the compensatory mechanism that maintains energy homeostasis for carriers of the variant. This might be accomplished by metabolomic studies in populations where the variant is rare (Caucasians) with those in which it is more frequent such as the Kenyan Masai population (MAF = 0.22).<sup>33</sup> It would also be important to see if there is a commensurate rise in CK-B levels for carriers of the *CKM* variant, and if the variants encoding them are related.

Since the variant confers no protective effect against the development of myalgia, this might provide a novel mechanistic rationale for a sub population of individuals presenting with statin intolerance or myalgia without raised CK levels.<sup>18</sup> Given the MAF of 0.02 of the Glu83Gly variant in *CKM*, approximately 4 in every 100 individuals is likely to be a carrier. With the rising number of statin users worldwide, CK monitoring for those complaining of adverse reactions is also likely to increase. The *CKM* Glu83Gly variant is therefore a key tool to be employed while determining the normalcy of CK test results.

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## Disclosures

No disclosures to be made

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## Tables

*Table 1. Association with baseline factors in the GoDARTS population with the CKM Glu83Gly genotype*

Variable	Glu83Gly carriers (n = 152)	Glu83Glu non-carriers (n = 4447)	P-value
Age (years) (mean, range)*	66 (38-90)	66 (27-93)	N.S.
Sex (% female)	46%	39%	N.S.
BMI (mean, range)*	29 (20-46)	30 (14-69)	N.S.
Type2 Diabetes (%)	74	72	N.S.
S-LDL mmol/L (mean, range)*	2.2 (0.74-5.7)	2.1 (0.23-6.5)	N.S.
S-HDL mmol/L (mean, range)*	1.3 (0.69,2.8)	1.3 (0.29,3.9)	N.S.
S-Creatinine $\mu$ mol/L (mean, range)*	94 (50-205)	92 (37-701)	N.S.

*BMI: Body Mass Index, LDL: Low-Density Lipoproteins, HDL: High-Density Lipoproteins*

*\*indicates that values were log transformed to achieve a normal distribution before associations were tested, S- indicates values measured in serum*

*Table 2. Creatine kinase levels by genotype in the GoDARTS cohort*

SNP	Mean CK (IU/L)	SD CK (IU/L)	Median CK (IU/L)	Minimum CK (IU/L)	Maximum CK (IU/L)	N
<b>rs11559024 (Glu83Gly)</b>						
T/T Glu83Glu	126	82	102	16	934	4447
T/C Glu83Gly	86	68	61	14	420	152



## Figure legends

*Fig 1* Forest plot of the meta-analysis of the association of creatine kinase with the CKM Glu83Gly variant in the GoDARTS study ( $p$  value =  $1 \times 10^{-16}$ ) and the JUPITER trial ( $p$  value =  $1 \times 10^{-45}$ ). Meta-analysis  $\beta$  = -0.18% (-0.20, -0.16)  $p$  value =  $6 \times 10^{-63}$

*Fig 2* Boxplot showing intra-individual variability demonstrated as standard deviation by Glu83Gly genotype. The reference line indicates the mean standard deviation in the population (40 IU/L).  $\beta$  = -0.24%  $p$  value =  $2 \times 10^{-5}$

*Fig 3* Association of the CKM Glu83Gly variant with the development of myalgia in the JUPITER trial. The effects are shown stratified by treatment allocation arm. Glu83Glu refers to homozygous carriers of the ancestral allele, while Glu83Gly refers of carriers of the variant

## Figures

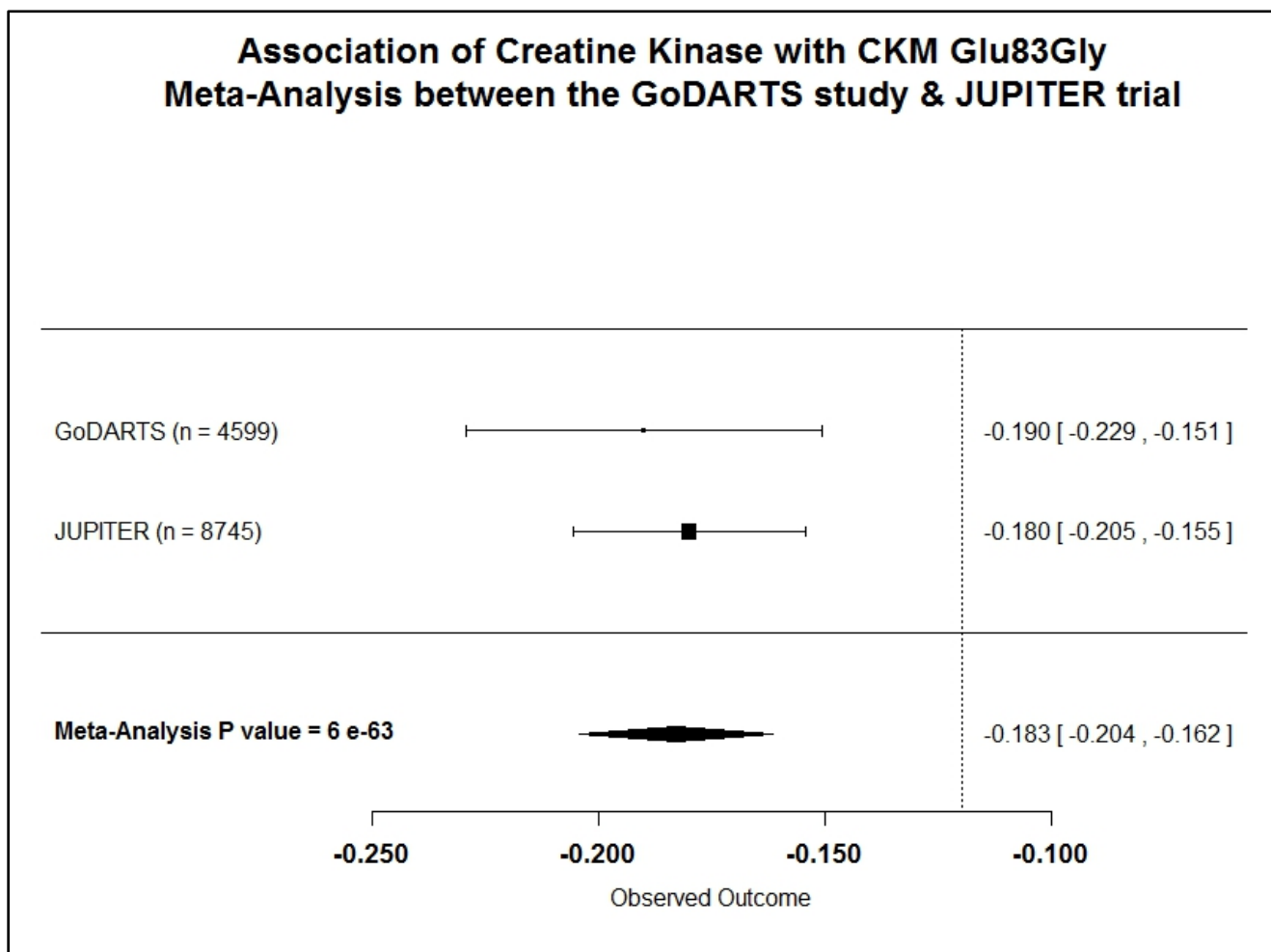


Figure 1

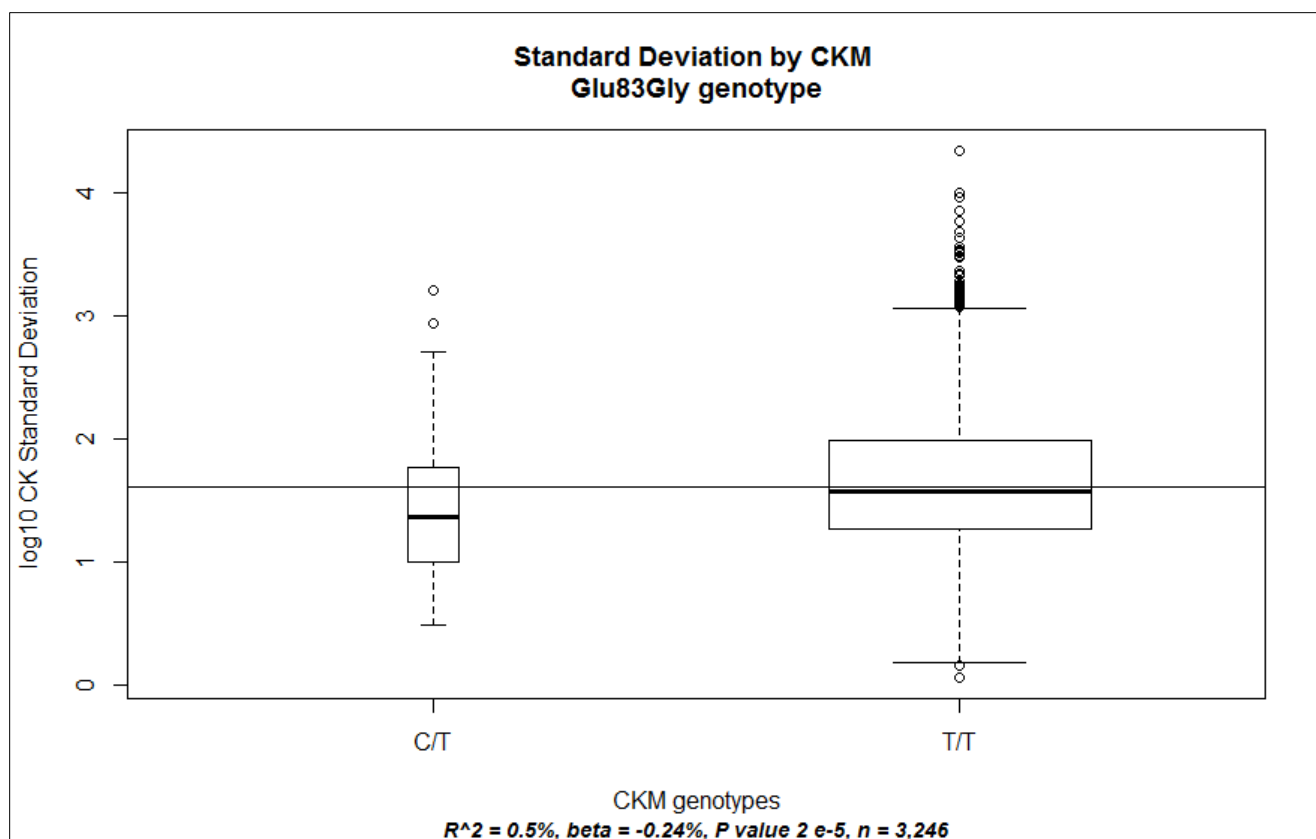


Figure 2

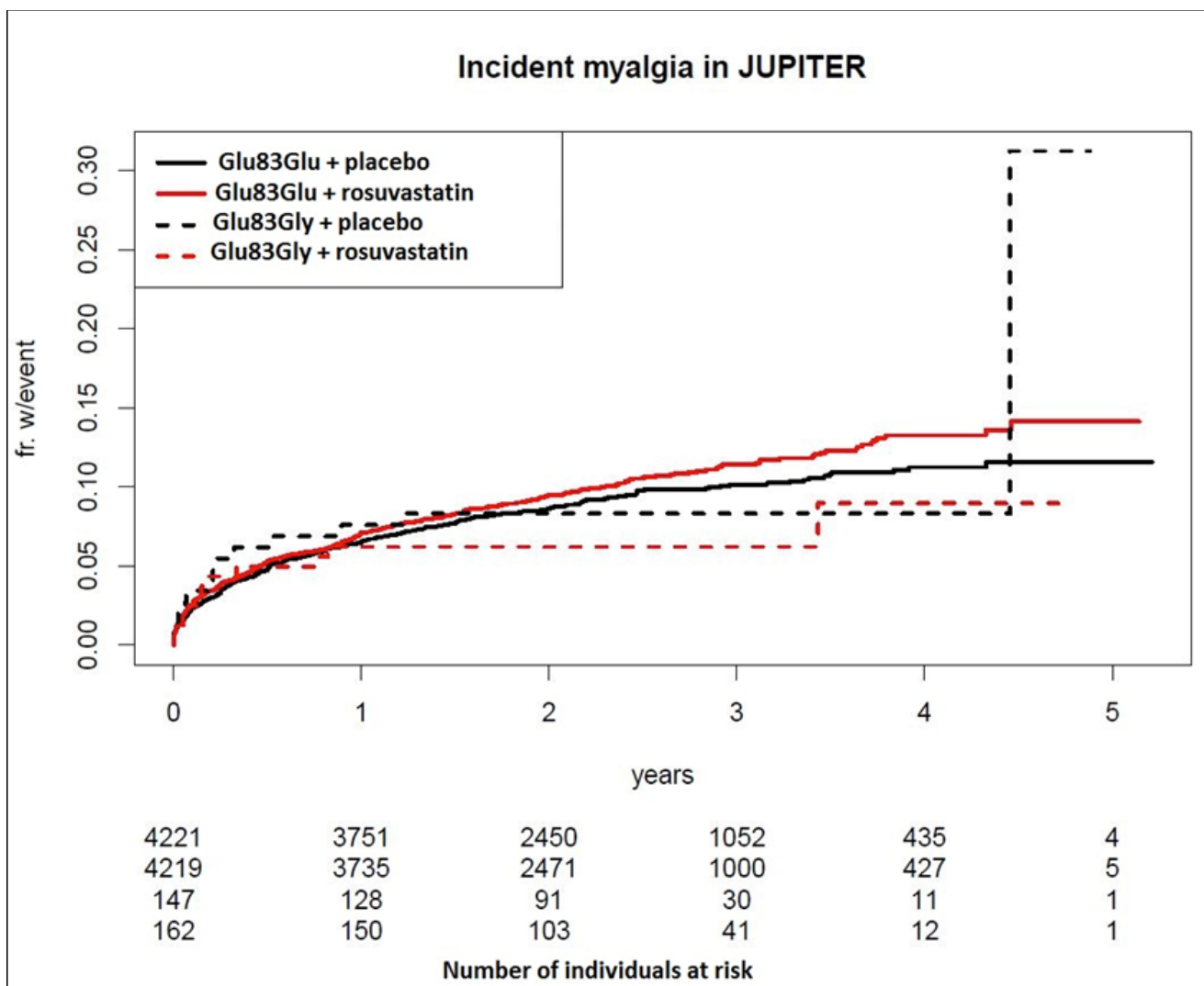


Figure 3